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(54) Title: NOVEL RECEPTORS OPG-2

(57) Abstract

Novel receptors in the TNF family: OPG-2.

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NOVEL RECEPTOR OPG-2

Background of the Invention:

The present invention relates to novel receptors in the TNF family. The novel receptors herein are referred to as OPG-2.

The TNF family consists of pairs of ligands and their specific receptors referred to as TNF family ligands and TNF family receptors (Bazzoni and Beutler, 1996). The family is involved in the regulation of the immune system and possibly other non-immunological systems. The regulation is often at a "master switch" level such that TNF family signaling can result in a large number of subsequent events best typified by TNF. TNF can initiate the general protective inflammatory response of an organism to foreign invasion that involves the altered display of adhesion molecules involved in cell trafficking, chemokine production to drive specific cells into specific compartments and the priming of various effector cells. As such, the regulation of these pathways has clinical potential.

The TNF receptor family is a collection of related proteins that generally consist of an extracellular domain, a transmembrane domain and an intracellular signaling domain. The extracellular domain is built from 2-6 copies of a tightly disulphide bonded domain and is recognized on the basis of the unique arrangement of cysteine residues. Each receptor binds to a corresponding ligand although one ligand may share several receptors. In some cases, it is clear that by alternate RNA splicing, soluble forms of the receptors lacking the transmembrane region and intracellular domain exist naturally. Moreover, in nature, truncated versions of these receptors exist and the soluble inhibitory form may have direct biological regulatory roles. Clearly, viruses have used this tactic to inhibit TNF activity in their host organisms (Smith, 1994). These receptors can signal a number of events including cell differentiation, cell death or cell survival signals. Cell death signaling often is triggered via relatively direct links to the caspase cascade of proteases e.g. Fas and TNF receptors. Most receptors in this class can also activate NF5 Kappa B controlled events.

OPG, a member of the TNF receptor superfamily has recently been identified. (Simonet et al., Osteoprotegerin: A Novel Secreted Protein Involved in the Regulation of Bone Density, Cell, Vol. 89 309-319, 1997., specifically incorporated herein by reference). OPG inhibits osteoclast maturation and protects bone from both normal

osteoclast remodeling and ovariectomy associated bone loss. This protein contains two characterized domains, see infra. It has been found that systemic delivery of OPG via the expression of rat or muring OPG transgenes in mice results in severe yet nonlethal osteopetrosis. The osteopetrotic phenotype caused by OPG overexpression differs significantly from those observed in other mouse osteopetrotic models, whether generated by the disruption of specific genes such as src or fos gene knockouts, or in naturally occurring mouse mutants.

The receptors are powerful tools to elucidate biological pathways via their easy conversion to immunoglobulin fusion proteins. These dimeric soluble receptor forms are good inhibitors of events mediated by either secreted or surface bound ligands. By binding to these ligands they prevent the ligand from interacting with cell associated receptors that can signal. Not only are these receptor-Ig fusion proteins useful in an experimental sense, but they have been successfully used clinically in the case of TNF-R-Ig to treat inflammatory bowel disease, rheumatoid arthritis and the acute clinical syndrome accompanying OKT3 administration (Eason et al., 1996; Feldmann et al., 1996; van Dullemen et al., 1995). One can envision that manipulation of the many events mediated by signaling through the TNF family of receptors will have wide application in the treatment of immune based diseases, such as AIDS, septic shock, cerebral malaria, graft rejection, cytoxicity, cachexia, apoptosis, antiviral response and inflammation and also the wide range of human diseases that have pathological sequelae due to immune system involvement. A soluble form of a recently described receptor, osteoprotegerin, can block the loss of bone mass and, therefore, the events controlled by TNF family receptor signaling are not necessarily limited to immune system regulation. Antibodies to the receptor can block ligand binding and hence can also have clinical application. Such antibodies are often very long-lived and may have advantages over soluble receptor-Ig fusion proteins which have shorter blood half-lives.

While inhibition of the receptor mediated pathway represents the most exploited therapeutic application of these receptors, originally it was the activation of the TNF receptors that showed clinical promise (Aggarwal and Natarajan, 1996). Activation of the TNF receptors can initiate cell death in the target cell and hence the application to tumors was and still is attractive (Eggermont et al., 1996). The receptor can be activated either by administration of the ligand, i.e. the natural pathway or some antibodies that can

crosslink the receptor are also potent agonists. Antibodies would have an advantage in oncology since they can persist in the blood for long periods whereas the ligands generally have short lifespans in the blood. As many of these receptors may be expressed more selectively in tumors or they may only signal cell death or differentiation in tumors, agonist antibodies could be good weapons in the treatment of cancer. Likewise, many positive immunological events are mediated via the TNF family receptors, e.g. host inflammatory reactions, antibody production etc. and therefore agonistic antibodies could have beneficial effects in other, non-oncological applications.

Paradoxically, the inhibition of a pathway may have clinical benefit in the treatment of tumors. For example the Fas ligand is expressed by some tumors and this expression can lead to the death of Fas positive lymphocytes thus facilitating the ability of the tumor to evade the immune system. In this case, inhibition of the Fas system could then allow the immune system to react to the tumor in other ways now that access is possible (Green and Ware, 1997).

The receptors are also useful to discover the corresponding ligand as they can serve as probes of the ligand in expression cloning techniques (Smith et al., 1993). Likewise, the receptors and ligands can form in vitro binding assays that will allow the identification of inhibitory substances. Such substances can form the basis of novel inhibitors of the pathways.

DETAILED DESCRIPTION

The present invention is related to a novel receptor designated OPG-2 which is a receptor to ligands of the TNF family.

A. Novel Receptors

OPG-2 is a novel receptor in the TNF family.

As used herein, the terms "OPG-2 receptor" refers to polypeptides having amino acid sequences which are substantially similar to the native mammalian OPG-2 receptor amino acid sequences, set forth in SEQ. ID. NO 2, and which are biologically active, as defined below, in that they are capable of binding to ligands or transducing a biological signal initiated by an OPG ligand binding to a cell, or cross- reacting with anti- OPG-2 antibodies raised against OPG-2. The terms as used herein include, but are not limited to, analogs or subunits of native proteins having at least 70-90% homology and which exhibit at least some biological activity in common with OPG-2, for example, shortened

constructs which retain the ability to bind to a ligand. Various bioequivalent protein and amino acid analogs are described in detail below.

The OPG-2 receptors of the invention may be isolated from mammalian tissues and purified to homogeneity, or isolated from cells which contain membrane-bound OPG-2, and purified to homogeneity. Methods for growing cells and isolating cell extracts are well know in the art, as are various cell types and growth and isolation methods. In general, any OPG-2 can be isolated from any cell or tissue expressing this protein using a cDNA probe, isolating mRNA and transcribing the mRNA into cDNA. Thereafter, the protein can be produced by inserting the cDNA into an expression vector, such as a virus, plasmid, cosmid or other expression vector, inserting the expression vector into a cell, and proliferating the resulting cells. The OPG-2 can then be isolated from the medium or cell extract by methods well known in the art. One skilled in the art can readily vary the vectors and cell lines and still obtain the claimed receptors.

Alternatively, OPG-2 may be chemically synthesized using the sequences set forth in SEQ.ID.NO.: 2, fragments or portions thereof.

OPG-2 is useful in the treatment of osteopenic disorders such as those characterized by excessive osteoclast activity such as primary osteoporosis, Paget's disease of the bone, hypercalcemia of malignancy and osteolytic metastases.

The present invention also encompasses DNA sequences which encode OPG-2. The DNA sequence is set forth in SEQ. ID. NO. 1 in comparison with one of two published sequences for OPG-1. In other embodiments, the invention relates to sequences that have at least 50% homology with DNA sequences encoding the C terminal receptor binding domain of the ligands and hybridize to the claimed DNA sequences or fragments thereof, and which encode OPG-2 having the sequences identified in SEQ. ID. NO. 2 or the activity of the protein encoded thereby.

The invention in certain embodiments furthermore relates to DNA sequences encoding OPG-2 where the sequences are operatively linked to an expression control sequence. Any suitable expression control sequences are useful in the claimed invention, and can easily be selected by one skilled in the art.

The invention also contemplates recombinant DNAs comprising a sequence encoding OPG-2 or fragments thereof, as well as hosts with stably integrated OPG-2 sequences introduced into their genome, or possessing episomal elements. Any suitable

host may be used in the invention, and can easily be selected by one skilled in the art without undue experimentation.

The claimed invention in certain embodiments encompasses recombinant OPG-2. One skilled in the art can readily isolate such recombinant receptors thereby providing substantially pure recombinant OPG-2 polypeptides. Isolated receptors of the invention are substantially free of other contaminating materials of natural or endogenous origin, and contain less than about 10- 15 % by mass of protein contaminants residual of production processes.

Mammalian Receptors within the scope of the invention also include, but are not limited to, primate, human, murine, canine, feline, bovine, ovine, equine and porcine OPG-2. Mammalian Receptors can also be obtained by cross species hybridization using a single stranded cDNA derived from the human OPG-2. DNA sequences of the invention can be used as a hybridization probe to isolate Receptor cDNAS from other mammalian cDNA libraries.

Derivatives of the Receptors within the scope of the invention also include various structural forms of the proteins of SEQ.ID.NO.: 2 which retain biological activity. For example, a receptor protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction.

OPG-2 derivatives may also be used as immunogens, reagents in a receptor-based immunoassay, or as binding agents for affinity purification procedures of OPG-2 ligands.

The present invention also includes OPG-2 with or without associated native-pattern glycosylation. One skilled in the art will understand that the glycosylation pattern on the receptor may vary depending on the particular expression system used. For example, typically, expression in bacteria such as E. coli results in a non-glycosylated molecule. OPG-2 derivatives may also be obtained by mutations of the receptors or their subunits. A mutant, as referred to herein, is a polypeptide homologous to a claimed Receptor but which has an amino acid sequence different from the native sequence due to a deletion, insertion or substitution.

Bioequivalent analogs of the Receptor proteins of the invention may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For

example, often cysteine residues can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. Other approaches to mutagenesis involve modifications, for example, to enhance expression in the chosen expression system.

Soluble Receptors of the invention may comprise subunits which have been changed. Thus, soluble peptides may be produced by truncating the polypeptide. Soluble Receptors of the invention may include any number of well-known leader sequences at the N-terminus. Such a sequence would allow the peptides to be expressed and targeted to the secretion pathway in a eukaryotic system.

The invention herein provides agents, such as agonists and antagonists, directed against the claimed receptors. In certain embodiments of this invention, the agent comprises a blocking agent that comprises an antibody directed against OPG-2 that inhibits OPG-2 pathway signaling. Preferably the antibody is a monoclonal antibody. Similarly, the claimed invention encompasses antibodies and other agents which act as agonists in the OPG-2 pathways.

Inhibitory anti- OPG-2 antibodies and other receptor blocking agents can be identified using screening methods that detect the ability of one or more agents either to bind to OPG-2, or ligands thereto, or to inhibit the effects of OPG-2 signaling on cells.

One skilled in the art will have knowledge of a number of assays that measure the strength of ligand-receptor binding and can be used to perform competition assays with putative OPG-2 blocking agents. The strength of the binding between a receptor and ligand can be measured using an enzyme-linked immunoadsorption assay (ELISA) or a radioimmunoassay (RIA). Specific binding may also be measured by flourescently labeling antibody-antigen complexes and performing fluorescence activated cell sorting analysis (FACS), or by performing other such immunodetection methods, all of which are techniques well-known in the art.

With any of these or other techniques for measuring receptor-ligand interactions, one skilled in the art can evaluate the ability of a blocking agent, alone or in combination with other agents, to inhibit binding of ligands to the receptor molecules. Such assays may also be used to test blocking agents or derivatives of such agents, i.e. fusions, chimeras, mutants or chemically altered forms, to optimize the ability of the agent to block receptor activation.

The receptor blocking agents of the invention in one embodiment comprise soluble OPG-2 molecules. Using the sequence information herein and recombinant DNA techniques well known in the art, functional fragments encoding the OPG-2 ligand binding domain can be cloned into a vector and expressed in an appropriate host to produce a modified receptor molecule. Soluble OPG-2 molecules that can compete with native OPG-2 for ligand binding according to the assays described herein can be selected as OPG-2 receptor blocking agents.

A soluble OPG-2 comprising amino acid sequences selected from those shown herein may be attached to one or more heterologous protein domains ("fusion domains") to increase the *in vivo* stability of the receptor fusion protein, or to modulate its biological activity or localization.

Preferably, stable plasma proteins -- which typically have a half-life greater than 20 hours in the circulation of a mammal-- are used to construct the receptor fusion proteins. Such plasma proteins include but are not limited to: immunoglobulins, serum albumin, lipoproteins, apolipoproteins and transferrin. Sequences that can target the soluble receptors to a particular cell or tissue type may also be attached to the receptor ligand binding domain to create a specifically localized soluble receptor fusion protein.

All or a functional fragment of OPG-2 comprising the OPG-2 ligand binding domain may be fused to an immunoglobulin constant region like the Fc domain of a human IgG1 heavy chain. Soluble receptor -IgG fusions proteins are common immunological reagents and methods for their construction are well known in the art. (see, e.g. U.S. Patent No. 5, 225, 538).

A functional OPG-2 ligand binding domain may be fused to an immunoglobulin (Ig) Fc domain derived from an immunoglobulin class or subclass other than IgG1. The Fc domains of antibodies belonging to different Ig classes or subclasses can activate diverse secondary effector functions. Activation occurs when the Fc domain is bound by a cognate Fc receptor. Secondary effector functions include the ability to activate the complement system, to cross the placenta and to bind various microbial proteins. The properties of the different classes and subclasses of immunoglobulins are described in the art.

Activation of the complement system initiates cascades of enzymatic reactions that mediate inflammation. The products of the complement system have a variety of

functions, including binding of bacteria, endocytosis, phagocytosis, cytotoxicity, free radical production and solubilization of immune complexes.

The complement enzyme cascade can be activated by the Fc domains of antigen-bound IgG1, IgG3 and Ig M antibodies. The Fc domain of IgG2 appears to be less effective, and the Fc domains of IgG4, IgA, IgD and IgE are ineffective at activating complement. Thus one can select an Fc domain based on whether its associated secondary effector functions are desirable for the particular immune response or disease being treated with the receptor-fusion protein.

It it would be advantageous to harm or kill the OPG-2 ligand bearing target cell, one could, for example, select an especially active Fc domain (IgG1) to make the fusion protein. Alternatively, if it would be desirable to target the OPG-2-FC fusion to a cell without triggering the complement system, an inactive IgG4 Fc domain could be selected.

Mutations in Fc domains that reduce or eliminate binding to Fc receptors and complement activation have been described in the art. These or other mutations can be used, alone or in combination to optimize the activity of the Fc domain used to construct the OPG-2-Fc fusion protein.

One skilled in the art will appreciate that different amino acid residues forming the junction point of the receptor-Ig fusion protein may alter the structure, stability and ultimate biological activity of the soluble OPG-2 fusion protein. One or more amino acids may be added to the C-terminus of the selected OPG-2 fragment to modify the junction point with the selected fusion domain.

The N-terminus of the OPG-2 fusion protein may also be varied by changing the position at which the selected OPG-2 DNA fragment is cleaved at its 5' end for insertion into the recombinant expression vector. The stability and activity of each OPG-2 receptor fusion protein may be tested and optimized using routine experimentation and the assays for selecting blocking agents described herein.

Using the OPG-2 binding domain sequences as shown herein, amino acid sequence variants may also be constructed to modify the affinity of the soluble OPG-2 molecules for their ligands. The soluble molecules of this invention can compete for binding with endogenous receptors. It is envisioned that any soluble molecule comprising an OPG-2 ligand binding domain that can compete with native receptors for

ligand binding is a receptor blocking agent that falls within the scope of the present invention.

In other embodiments of this invention, antibodies directed against OPG-2 (anti-OPG-2 abs) function as receptor blocking agents. The antibodies of this invention can be polyclonal or monoclonal and can be modified to optimize their ability to block OPG-2 signaling, their bioavailability, stability or other desired traits.

Polyclonal antibody sera directed against OPG-2 are prepared using conventional techniques by injecting animals such as goats, rabbits, rats, hamsters or mice subcutaneously with OPG-2, or an OPG-2 -Fc fusion protein in Freund's adjuvant, followed by booster intraperitoneal or subcutaneous injection in incomplete Freund's. Polyclonal antisera containing the desired antibodies directed against OPG-2 can then be screened by conventional immunological procedures.

Various forms of anti-OPG-2 abs can also be made using standard recombinant DNA techniques. For example, "chimeric" antibodies can be constructed in which the antigen binding domain from an animal antibody is linked to a human constant domain. Chimeric antibodies reduce the observed immunogenic responses elicited by animal antibodies when used in human clinical treatments.

In addition, recombinant "humanized" antibodies which can recognize OPG-2 can be synthesized. Human antibodies are chimeras comprising mostly human IgG sequences into which the regions responsible for specific antigen-binding have been inserted. (e.g. WO 94/04679). Animals are immunized with the desired antigen, the corresponding antibodies are isolated, and the portion of the variable region sequences responsible for specific antigen binding are removed. The animal-derived antigen binding regions are then cloned into the appropriate position of human antibody genes in which the antigen binding regions have been deleted. Humanized antibodies minimize the use of heterologous (inter species) sequences in human antibodies, and are less likely to elicit immune responses in the human being treated.

Construction of different classes of recombinant anti-OPG-2 antibodies can also be accomplished by making chimeric or humanized antibodies comprising the anti-OPG-2 variable domains and human constant domains isolated from different classes of immunoglobulins. For example, anti OPG-2 antibodies with increased antigen binding

site valencies can be recombinantly produced by cloning the antigen binding site into vectors carrying the human μ chain constant regions.

In addition, standard recombinant DNA techniques can be used to alter the binding affinities of recombinant antibodies with their antigens by altering amino acid residues in the vicinity of the antigen binding sites. The antigen binding affinity of a humanized antibody can be increased by mutagenesis based on molecular modeling.

It may be desirable to increase or decrease the affinity of anti-OPG-2 antibodies for the receptors depending on the targeted tissue type or the particular treatment schedule envisioned. For example, it may be advantageous to treat a patient with constant levels of anti-Receptor antibodies with reduced ability to signal through the pathway for semi-prophylactic treatments. Likewise, inhibitory anti-OPG-2 antibodies with increased affinity for the receptors may be advantageous for short term treatments. Additionally, such antibodies can be used in diagnostic assays.

The claimed invention in yet other embodiments encompasses pharmaceutical compositions comprising an effective amount of OPG-2 blocking or activating agent, and pharmaceutically acceptable carriers. The compositions of the invention will be administered at an effective dose to treat the particular clinical condition addressed. Determination of a preferred pharmaceutical formulation and a therapeutically efficient dose regimen for a given application is well within the skill of the art taking into consideration for example, the condition and weight of the patient, the extent of desired treatment and the tolerance of the patient for the treatment. Doses of about 1 mg/kg of a soluble OPG-2 are expected to be suitable starting points for optimizing treatment dosages.

Determination of a therapeutically effective dose can also be assessed by performing *in vitro* experiments that measure the concentration of the blocking or activating agent. The binding assays described herein are useful, as are other assays known in the art.

Administration of the soluble activating or blocking agents of the invention, alone or in combination, including isolated and purified forms, their salts, or pharmaceutically acceptable derivatives thereof may be accomplished using any of the conventionally accepted modes of administration of agents which exhibit immunosuppressive activity.

EXAMPLES:

Generation of Soluble Receptor Forms:

To form an receptor inhibitor for use in man, one requires the human receptor cDNA sequence of the extracellular domain. If the mouse form is known, human cDNA libraries can be easily screened using the mouse cDNA sequence and such manipulations are routinely carried out in this area. With a human cDNA sequence, one can design oligonucleotide primers to PCR amplify the extracellular domain of the receptor in the absence of the transmembrane and intracellular domains. Typically, one includes most of the amino acids between the last disulfide linked "TNF domain" and the transmembrane domain. One could vary the amount of "stalk" region included to optimize the potency of the resultant soluble receptor. This amplified piece would be engineered to include suitable restriction sites to allow cloning into various C-terminal Ig fusion chimera vectors. Alternatively, one could insert a stop signal at the 3'end and make a truncated form of the receptor without resorting to the use of a Ig fusion chimera approach. The resultant vectors can be expressed in most systems used in biotechnology including yeast, insect cells, bacteria and mammalian cells and examples exist for all types of expression. Various human Fc domains can be attached to optimize or eliminate FcR and complement interactions as desired. Alternatively, mutated forms of these Fc domains can be used to selectively remove FcR or complement interactions or the attachment of N-linked sugars to the Fc domain which has certain advantages. Since OPG-2 is already a soluble receptor it may be possible to simply express the native form of the protein.

Generation of Agonistic or Antagonistic Antibodies:

The above described soluble receptor forms can be used to immunize mice and to make monoclonal antibodies by conventional methods. The resultant mAbs identified by ELISA methods can be further screened for agonist activity either as soluble antibodies or immobilized on plastic in various in vitro cellular assays. Often the death of the HT29 cell line is a convenient system that is sensitive to signalling through many TNF receptors. If this line does not possess the receptor of interest, that full length receptor can be stably transfected into the HT29 line to now allow the cytotoxicity assay to work.

Alternatively, such cells can be used in the Cytosensor apparatus to assess whether activation of the receptor can elicit a pH change that is indicative of a signalling event. TNF family receptors signal well in such a format and this method does not require one to know the actual biological events triggered by the receptor. The agonistic mAbs would be "humanized" for clinical use. This procedure can also be used to define antagonistic mAbs. Such mAbs would be defined by the lack of agonist activity and the ability to inhibit receptor-ligand interactions as monitored by ELISA, classical binding or BIAcore techniques.

Screening for Inhibitors of the Receptor-Ligand Interaction:

Using the receptor-Ig fusion protein, one can screen other combinatorial libraries for molecules that can bind the receptor directly. These molecules can then be tested in an ELISA formatted assay using the receptor-Ig fusion protein and a soluble form of the ligand for the ability to inhibit the receptor-ligand interaction. This ELISA can be used directly to screen various natural product libraries etc. for inhibitory compounds. The receptor can be transfected into a cell line such as the HT29 line to form a biological assay (in this case cytotoxicity) that can then form the screening assay.

It will be apparent to those skilled in the art that various modifications and variations can be made in the polypeptides, compositions and methods of the invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided that they come within the scope of the appended claims and their equivalents.

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WE CLAIM:

1. A polypeptide comprising an amino acid sequence encoded by a DNA sequence comprising SEQ ID. NO. 1.
2. The polypeptide of claim 1 wherein said polypeptide is soluble.
3. The polypeptide of claim 1 wherein said polypeptide is modified to alter the selectivity or potency of the polypeptide.
4. A DNA sequence encoding OPG-2, or a biologically active fragment thereof, said DNA sequence comprising SEQ.ID. NO. 1.
5. A DNA sequence that hybridizes to at least a fragment of SEQ. ID. NO. 1, said fragment comprising at least 20 consecutive bases, said DNA encoding a polypeptide that is at least 75% homologous with an active site of OPG-2.
6. A recombinant DNA molecule comprising a DNA sequence encoding OPG-2, said sequence operatively linked to an expression control sequence.
7. A unicellular host transformed with a recombinant DNA molecule of claim 4.
8. A unicellular host transformed with a recombinant DNA molecule of claim 5 or claim 6.
9. A method for producing substantially pure OPG-2 comprising culturing the unicellular host of claim 7.
10. A method for producing substantially pure OPG-2 comprising culturing the unicellular host of claim 8.
11. A pharmaceutical composition comprising a therapeutically effective amount of anti-OPG-2 antibodies, and a pharmaceutically acceptable carrier.
12. A method for preventing, reducing the severity of an immune response comprising administering a therapeutically effective amount of a pharmaceutical composition according to claim11.
13. A method for treating cancer comprising administering a therapeutically effective amount of a pharmaceutical composition according to claim 11.
14. A method for identifying a ligand to OPG-2 comprising the steps of (a) providing OPG-2 or a fragment thereof; (b) labeling said receptor or fragment thereof with a detectable label; (c) screening to detect ligands which bind to the detectably labeled OPG-2 or fragment thereof.

15. A method for producing an antibody preparation reactive to OPG-2 or biologically active fragments thereof comprising the step of immunizing an organism with said receptor or biologically active fragments thereof.
16. An antibody preparation reactive to OPG-2 or biologically active fragments thereof.
17. A pharmaceutical composition comprising an antibody preparation of claim 16 and a pharmaceutically acceptable carrier.
18. A method of expressing a gene in a mammalian cell comprising the steps of: (a) introducing DNA encoding OPG-2 or biologically active fragments thereof into a cell; (b) allowing said cell to live under conditions such that the gene is expressed.
19. A method for treating or reducing the advancement, severity or effects of a disease in a mammal comprising the step of administering a pharmaceutical composition which comprises a therapeutically effective amount of an OPG-2 blocking agent and a pharmaceutically acceptable carrier.
20. The method of claim 19 wherein the blocking agent is selected from the group consisting of a soluble OPG-2, and antibody directed against OPG-2.
21. The method according to claim 19 wherein the mammal is a human.
22. A soluble OPG-2 fusion protein comprising a human immunoglobulin FC domain.

SEQ ID NO:1

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   1  GGCACGAGCT GACCACTGCA AAGCCAGAGG ACTTCCCCCT GCTGCACAGG
  51  TTCAGCATGT TTGTGCGTCC ACACCACAAG CAGCGCTTCT CACAGACGTG
 101  CACAGACCTG ACCGGCCGGC CCTACCCGGG CATGGAGCCA CCGGGACCCC
 151  AGGAGGAGAG GCTTGCCGTG CCTCCTGTGC TTACCCACAG GGCTCCCCAA
 201  CCAGGCCCCT CACGGTCCGA GAAGACCGGG AAGACCCAGA GCAAGATCTC
 251  GTCCTTCCTT AGACAGAGGC CAGCAGGGAC TGTGGGGGCG GGCGGTGAGG
 301  ATGCAGGTCC CAGCCAGTCC TCAGGACCTC CCCACGGGCC TGCAGCATCT
 351  GAGTGGGGCC TCTAGGATGT GCCCAGCCTG CCACACCGCC TCCAGGAAGC
 401  AGAGCGTCAT GCAGGTCTTC TGGCCGGAGC CCCACAAGGA CCATGAGGGC
 451  GCTGGAGGGG CCAGGCCTGT CGCTGCTGTG CCTGGTGTTG GCGCTGCCTG
 501  CCCTGCTGCC GGTGCCGGCT GTACGCGGAG TGGCAGAAAC ACCCACCTAC
 551  CCCTGGCGGG ACGCAGAGAC AGGGGAGCGG CTGGTGTGCG CCCAGTGCCC
 601  CCCAGGCACC TTTGTGCAGC GGCCGTGCCG CCGAGACAGC CCCACGACGT
 651  GTGGCCCGTG TCCACCGCGC CACTACACGC AGTTCTGGAA CTACCTGGAG
 701  CGCTGCCGCT ACTGCAACGT CCTCTGCGGG GAGCGTGAGG AGGAGGCACG
 751  GGCTTGCCAC GCCACCCACA ACCGTGCCTG CCGCTGCCGC ACCGGCTTCT
 801  TCGCGCACGC TGGTTTCTGC TTGGAGCACG CATCGTGTCC ACCTGGTGCC
 851  GGCGTGATTG CCCCGGGCAC CCCCAGCCAG AACACGCAGT GCCAGCCGTG
 901  CCCCCCAGGC ACCTTCTCAG CCAGCAGCTC CAGCTCAGAG CAGTGCCAGC
 951  CCCACCGCAA CTGCACGGCC CTGGGCCTGG CCCTCAATGT GCCAGGCTCT
1001  TCCTCCCATG ACACCCTGTG CACCAGCTGC ACTGGCTTCC CCCTCAGCAC
1051  CAGGGTACCA GGAGCTGAGG AGTGTGAGCG TGCCGTCATC GACTTTGTGG
1101  CTTTCCAGGA CATCTCCATC AAGAGGCTGC AGCGGCTGCT GCAGGCCCTC
1151  GAGGCCCCGG AGGGCTGGGG TCCGACACCA AGGGCGGGCC GCGCGGCCTT
1201  GCAGCTGAAG CTGCGTCGGC GGCTCACGGA GCTCCTGGGG GCGCAGGACG
1251  GGGCGCTGCT GGTGCGGCTG CTGCAGGCGC TGCGCGTGGC CAGGATGCCC
1301  GGGCTGGAGC GGAGCGTCCG TGAGCGCTTC CTCCCTGTGC ACTGATCCTG
1351  GCCCCCTCTT ATTTATTCTA CATCCTTGGC ACCCCACTTG CACTGAAAGA
1401  GGCTTTTTTT TAAATAGAAG AAATGAGGTT TCTTAAAGCT TATTTTTATA
1451  AAGCTTTTTC AT
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SEQ ID NO: 2

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   1  MRALEGPGLS LLCLVLALPA LLPVPAVRGV AETPTYPWRD AETGERLVCA
  51  QCPPGTFVQR PCRRDSPTTC GPCPPRHYTQ FWNYLERCRY CNVLCGEREE
 101  EARACHATHN RACRCRTGFF AHAGFCLEHA SCPPGAGVIA PGTPSQNTQC
 151  QPCPPGTFSA SSSSSEQCQP HRNCTALGLA LNVPGSSSHD TLCTSCTGFP
 201  LSTRVPGAEE CERAVIDFVA FQDISIKRLQ RLLQALEAPE GWGPTPRAGR
 251  AALQLKLRRR LTELLGAQDG ALLVRLLQAL RVARMPGLER SVRERFLPVH
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INTERNATIONAL SEARCH REPORT

national Application No
PCT/US 98/25065

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/705 C12N15/12 A61K39/395 C07K16/28 A61K38/17
C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 30694 A (HUMAN GENOME SCIENCES INC ;FENG PING (US); NI JIAN (US); EBNER REI) 16 July 1998 see figure 1 ---	1-18, 20-22
P,X	EP 0 861 850 A (SMITHKLINE BEECHAM CORP) 2 September 1998 see figure 1 ---	1-18, 20-22
E	WO 99 04001 A (ZYMOGENETICS INC) 28 January 1999 see figure 1 ---	1-18, 20-22
E	WO 99 07738 A (MASIAKOWSKI PIOTR J ;PROCTER & GAMBLE COMPAGNY (US); MORRIS JODI () 18 February 1999 see sequences --- -/--	1-18, 20-22

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 1999	20.04.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bilang, J

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

national Application No
PCT/US 98/25065

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SIMONET W ET AL: "Osteoprotegerin: a novel secreted protein involved in the regulation of bone density" CELL, no. 89, 18 April 1997, page 309 309 XP002077048 cited in the application see the whole document -----	1-18, 20-22

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/25065

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 12, 13, 20, and 21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 19
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

Claim 19 covers a method comprising the step of administering an OPG-2 blocking agent.
No search can be carried out for substances that are only characterized by their effect.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No
PCT/US 98/25065

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9830694 A	16-07-1998	AU 5815798 A	03-08-1998
		AU 6238698 A	03-08-1998
		WO 9830693 A	16-07-1998
EP 0861850 A	02-09-1998	CA 2220852 A	03-08-1998
		JP 10215886 A	18-08-1998
WO 9904001 A	28-01-1999	NONE	
WO 9907738 A	18-02-1999	NONE	